OCCURRENCE OF *Alternaria* spp. IN THE SEEDS OF BASIL AND ITS PATHOGENICITY

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SUMMARY

Eighteen seed samples of basil, belonging to the cultivars most frequently grown in the Piedmont area for pesto production and obtained from farms affected by the disease in Piedmont during the fall of 2010 as well as from experimental lines of basil, were assayed for the presence of *Alternaria* spp., the causal agent of leaf spot of basil. Isolations were carried out by disinfected and not disinfected seeds. All eighteen tested samples resulted contaminated by *Alternaria* spp. and the frequency of isolation of *Alternaria* spp. colonies was higher in the case of not disinfected seeds for all samples tested. In the case of seeds belonging to experimental lines of basil, the frequency of isolation of *Alternaria* spp. was 1.18% from not disinfected seeds, and 0.43% from disinfected seeds. All eighteen tested samples resulted contaminated by *Alternaria* spp. out of 32 obtained by disinfected seeds were not virulent. Only two isolates of *Alternaria* spp. obtained from seeds of experimental lines of basil under development were tested for their virulence, 22 were able to infect basil leaves. Twenty of them, obtained from disinfected and not disinfected seeds, showed a good level of virulence, infecting more than 30% of leaves. One hundred eighty one isolates of *Alternaria* spp. strains were obtained from seeds of commercial varieties of basil, *Alternaria* spp. was isolated respectively from 7.29% and 2.62% of not disinfected and disinfected seeds. When twenty-eight isolates of *Alternaria* spp. obtained from seeds of experimental lines of basil were tested for virulence, 22 were able to infect basil leaves. Twenty of them, obtained from disinfected and not disinfected seeds, showed a good level of virulence, infecting more than 30% of leaves. One hundred eighty one isolates of *Alternaria* spp. strains were obtained from seeds of commercial varieties, of which 149 from not disinfected seeds and 32 from disinfected seeds. Out of 149 strains obtained from not disinfected seeds, 102 were virulent and 47 were not virulent. Only two isolates of *Alternaria* spp. out of 32 obtained by disinfected seeds were not virulent when tested on basil. This work provides evidence that *Alternaria* spp. the causal agent of leaf spot of basil, is seed-transmitted, which suggests that seeds may be important in disseminating the pathogen.

Key words: Alternaria leaf spot, seed transmission, virulence.

INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) is an economically important herb crop in several Mediterranean countries. Although the overall acreage is limited to a few hundreds of hectares, the crop is quite popular and strongly characterizes the Mediterranean cuisine. It is used as fresh, dried, and processed for flavouring and fragrances and in traditional medicine. Approximately 170 ha are grown annually in Italy under greenhouse conditions (ISTAT, 2009), where the cv. Genovese gigante is the most popular, with an increase of soilless growing systems. Although basil production is mostly widespread in the Liguria Region, its cultivation increased during the past few years in Piedmont, where such crop is grown in relatively big farms for pesto production. Basil is susceptible to several diseases, reviewed by Garibaldi *et al.* in 1997. More recently, new diseases such as leaf spots caused by *Corynespora cassicola* (Garibaldi *et al.*, 2007) and downy mildew, caused by *Peronospora belbahrii* have been reported in several countries as well as Italy (Lefort *et al.*, 2003; Garibaldi *et al.*, 2004a).

During the summer-fall 2010, extensive necrosis were observed on leaves of sweet basil plants, grown in soilless systems as well as in soil in Piedmont (northern Italy). The disease, caused by *Alternaria alternata*, affected 10% of 60-day-old soilless-grown plants and 40% of 5-month-old plants grown in soil. The first symptoms were usually lesions 1-50 mm in diameter which progressively turned black. Lesions usually started on the upper side of older leaves at the leaf margins and tips and showed a yellow halo. Severely affected plants were defoliated. Infected plants rarely died, but the presence of lesions reduced their commercial value (Garibaldi *et al.*, 2011). Taba *et al.* (2009) showed that the black lesion of basil grown in greenhouse in Japan were caused by *Alternaria alternata*. Recently in Israel, a similar black spot caused by *Alternaria* sp. was observed at the harvesting of summer basil (Kenigsbuch *et al.*, 2010).

Circumstantial evidence from surveys in the area interested by the disease suggested that the sudden appearance of this disease in many farms was possibly due to the transmission of the pathogen by seeds.

The present study was undertaken to ascertain the
Seeds as vehicles for Alternaria leaf spot of basil

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extent of and the variation in occurrence of Alternaria spp. in basil seeds, and the pathogenicity of the isolates obtained from that seeds.

MATERIAL AND METHODS

Seed infection evaluation. Eleven seed samples of basil, belonging to the cultivars most frequently grown in the Piedmont area for pesto production (Table 1) obtained from farms affected by the disease in Piedmont during the fall of 2010, and seven seed samples from experimental line of basil were assayed for the presence of Alternaria spp. on a medium containing Potato dextrose agar (PDA, Difco, Detroit, Michigan, USA) amended with 25 mg l⁻¹ of streptomycin sulphate.

<table>
<thead>
<tr>
<th>Seed sample code</th>
<th>Cultivar</th>
<th>Origin</th>
<th>Seed company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bi 29257</td>
<td>Basilico italiano</td>
<td>Experimental lines</td>
<td>Olter</td>
</tr>
<tr>
<td>1/11</td>
<td>Genovese Superbo FT</td>
<td>Commercial</td>
<td>Sais</td>
</tr>
<tr>
<td>2/11</td>
<td>Genovese Gecom FT</td>
<td>Commercial</td>
<td>Sais</td>
</tr>
<tr>
<td>3/11</td>
<td>Genovese AN 4055 (biologico)</td>
<td>Commercial</td>
<td>Anseme</td>
</tr>
<tr>
<td>4/11</td>
<td>Genovese ISI 602</td>
<td>Commercial</td>
<td>ISI Sementi</td>
</tr>
<tr>
<td>5/11</td>
<td>Genovese Italico FT</td>
<td>Commercial</td>
<td>La Semiorto Sementi</td>
</tr>
<tr>
<td>6/11</td>
<td>Genovese B/580</td>
<td>Commercial</td>
<td>Pagano Costantino</td>
</tr>
<tr>
<td>7/11</td>
<td>Genovese D9</td>
<td>Commercial</td>
<td>Olter</td>
</tr>
<tr>
<td>8/11</td>
<td>Genovese RCS TC7X</td>
<td>Commercial</td>
<td>Four</td>
</tr>
<tr>
<td>9/11</td>
<td>Basilico Aromatico Ligure</td>
<td>Commercial</td>
<td>Semacoop</td>
</tr>
<tr>
<td>10/11</td>
<td>Mammolo</td>
<td>Commercial</td>
<td>Semacoop</td>
</tr>
<tr>
<td>11/11</td>
<td>Profumo</td>
<td>Commercial</td>
<td>Semacoop</td>
</tr>
</tbody>
</table>

Table 2. Evaluation of the presence of Alternaria spp. on basil seeds obtained from experimental lines of basil not yet commercial (Trial 1).

<table>
<thead>
<tr>
<th>Seed sample</th>
<th>Number of Alternaria colonies detected on (Isolate code)</th>
<th>400 seeds tested not disinfected</th>
<th>400 seeds tested disinfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>BASG</td>
<td>1 (BASG-1NL)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1BA</td>
<td>1 (1BA-3NL; 1BA-4NL; 1BA-18NL; 1BA-19NL; 1BA-23NL; 1BA-27NL; 1BA-24NL; 1BA-16NL; 1BA-17NL)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bi</td>
<td>2 (Bi-10NL; Bi-11NL)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RCS</td>
<td>1 (RCS-16NL)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5BA</td>
<td>3 (5BA-10NL; 5BA-12NL)</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Bi29257</td>
<td>15 (Bi 29257-3NL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIO3255</td>
<td>2 (BIO3255-1NL; BIO3255-2NL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>33 (1.18%)</td>
<td>12 (0.45%)</td>
<td></td>
</tr>
</tbody>
</table>
Subsamples represented by 400 seeds (disinfected or not) were tested on Petri plates (10 seeds/plate) in two trials. In trial 1, the experimental lines of basil coded BASG, 1BA, Bi, RCS, 5BA, Bi29257, BIO 3255 were used, while in trial 2, the commercial seed samples 1/11, 2/11, 3/11, 4/11, 5/11, 6/11, 7/11, 8/11, 9/11, 10/11, 11/11 were tested.

Isolations were made from seeds either non disinfected or surface disinfected for 1 min in 1% sodium hypochlorite solution, washed in sterile water for 5 min and dried under a sterile hood. The Petri dishes were incubated at 22°C in 12 h light and 12 h darkness at 75% R.H. for 7-10 days. Seeds infected by *Alternaria* spp. were surrounded by characteristic fast-growing, gray-brown fungal colonies. Identification was carried out by conidial morphology (Mathur and Kongsdal, 2003).

**Isolates used and their preservation.** The 549 isolates obtained from seeds were coded as reported under Tables 2 and 3. The *Alternaria alternata* isolate Altbas. 1/10 (GenBank Accession number HQ540552) ob-

<table>
<thead>
<tr>
<th>Seed sample</th>
<th>400 seeds tested not disinfected</th>
<th>400 seeds tested disinfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/11</td>
<td>34</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>(1/11-1NL; 1/11-2NL; 1/11-3NL; 1/11-4NL; 1/11-5NL; 1/11-6NL; 1/11-7NL; 1/11-8NL; 1/11-9NL; 1/11-10NL; 1/11-11NL; 1/11-12NL; 1/11-13NL; 1/11-14NL; 1/11-15NL; 1/11-16NL; 1/11-17NL; 1/11-18NL; 1/11-19NL; 1/11-20NL; 1/11-21NL; 1/11-22NL; 1/11-23NL; 1/11-24NL; 1/11-25NL; 1/11-26NL; 1/11-30NL; 1/11-31NL; 1/11-32NL; 1/11-35NL; 1/11-40NL; 1/11-50NL; 1/11-51NL; 1/11-52NL)</td>
<td>(1/11-2L; 1/11-3L; 1/11-13L; 1/11-15L; 1/11-16L; 1/11-19L; 1/11-23L; 1/11-25L; 1/11-32L; 1/11-33L)</td>
</tr>
<tr>
<td>2/11</td>
<td>52</td>
<td>5</td>
</tr>
<tr>
<td>3/11</td>
<td>62</td>
<td>16</td>
</tr>
<tr>
<td>4/11</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(4/11-1NL; 4/11-2NL)</td>
<td>(4/11-1L)</td>
</tr>
<tr>
<td>5/11</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>(5/11-1NL; 5/11-2NL; 5/11-3NL; 5/11-4NL; 5/11-6NL; 5/11-7NL)</td>
<td>(5/11-1L; 5/11-2L; 5/11-8L; 5/11-9L; 5/11-10L)</td>
</tr>
<tr>
<td>6/11</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(6/11-9NL; 6/11-13NL)</td>
<td></td>
</tr>
<tr>
<td>7/11</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(7/11-10NL)</td>
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<tr>
<td>8/11</td>
<td>165</td>
<td>73</td>
</tr>
<tr>
<td>9/11</td>
<td>27</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>(9/11-4NL; 9/11-5NL; 9/11-7NL; 9/11-8NL; 9/11-33NL)</td>
<td>(9/11-18L)</td>
</tr>
<tr>
<td>10/11</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>(10/11-1NL; 10/11-2NL; 10/11-3NL; 10/11-4NL)</td>
<td>(10/11-1L; 10/11-2L)</td>
</tr>
<tr>
<td>11/11</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(11/11-8NL; 11/11-13NL; 11/11-14NL)</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>379 (7.29%)</td>
<td>125 (2.62%)</td>
</tr>
</tbody>
</table>
Inoculum production and pathogenicity test. The different strains of putative isolates of *Alternaria* spp. were grown on V8 in a growth chamber in darkness at 22-24°C for two weeks.

For the pathogenicity test, basil seeds cv. Genovese gigante (Furia sementi) were sown into a steamed potting soil mix (peat: composted broadleaf bark: clay, 60:20:20 v/v) in plastic pots (2 l capacity) and maintained at 22°C, with 12 h/day of fluorescent light. Thirty-day old plants were artificially inoculated by using mycelium fragments removed using a sterilized cork borer from 15-day-old cultures. Thirty plants of basil were inoculated with each of the strains obtained from seeds. Uninoculated plants were prepared similarly but sprayed with deionised water. Plants were covered with plastic bags for 5 days. Six trials were carried out in a growth chamber at 24±1°C, under 12 h/day fluorescent light: one trial concerned isolates of *Alternaria* spp. from seeds of experimental lines (Table 4), while 5 trials were carried out with isolates from seeds of commercial varieties (Table 5). Plants were checked two weeks after inoculation for disease development by evaluating the number of infected leaves per plant.

### RESULTS AND DISCUSSION

All eighteen tested seed samples resulted contaminated by *Alternaria* spp., as shown in Tables 2 and 3. The frequency of isolation of *Alternaria* spp. colonies was higher in the case of not disinfected seeds for all samples tested. For instance, in the case of seeds belonging to experimental lines of basil, the frequency of isolation of *Alternaria* spp. from seeds was 1.18% for not disinfected seeds and 0.43% for disinfected seeds (Table 2). In the case of seeds belonging to commercial varieties of basil, *Alternaria* spp. was isolated respectively from 7.29% and 2.62% of not disinfected and disinfected seeds (Table 3).

When 28 isolates of *Alternaria* spp. obtained from seeds of experimental lines of basil under development were tested for their virulence, 22 were able to infect basil leaves, 20 of them, obtained from disinfected and not disinfected seeds, showed high level of virulence, infecting more than 30% of leaves (Table 4).

One hundred eighty one isolates of *Alternaria* spp. strains were obtained from seeds of commercial varieties, of which 149 from not disinfected seeds and 32 from disinfected seeds. Out of 149 strains obtained from not disinfected seeds, 102 were virulent and 47 were not virulent (Table 5). Only two isolates of *Alternaria* spp. out of 32 obtained by disinfected seeds were not virulent when tested on basil (Table 5).

This work provides evidence that *Alternaria* spp., the causal agent of leaf spot of basil, is seed-transmitted, which suggests that seeds may be important in disseminating the pathogen. The results of this study do not provide information on the effects of *Alternaria* spp. on the quality and germination ability of basil seeds, but do indicate that the seeds are a potential source of inoculum for development of Alternaria leaf spot. Neergarad (1977) listed a long series of *Alternaria* spp., including *A. alternata* on tobacco and sugar beet, *A. brassica* and *A. brassicicola* on brassicas, *A. porri* on onion, *A. dauci* on carrot, that impair the germination of seeds and cause damping-off diseases.

The fact that not all the isolates of *Alternaria* spp. obtained during fall-winter 2010 from infected leaves of basil cv. Genovese (Four sementi), grown in commercial field in Piedmont (northern Italy) was used as control. The different strains were maintained on PDA at 8°C.
## Table 5.

<table>
<thead>
<tr>
<th>Trial 2</th>
<th>From seeds</th>
<th>% infected leaves</th>
<th>Sample code</th>
<th>From seeds</th>
<th>% infected leaves</th>
<th>Sample code</th>
<th>From seeds</th>
<th>% infected leaves</th>
<th>Sample code</th>
<th>From seeds</th>
<th>% infected leaves</th>
<th>Sample code</th>
<th>From seeds</th>
<th>% infected leaves</th>
<th>Sample code</th>
<th>From seeds</th>
<th>% infected leaves</th>
<th>Sample code</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/11-1NL Not disinfected 40.0 be</td>
<td>5/11-8L Disinfected</td>
<td>63.3 ei</td>
<td>8/11-6NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>1/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>8/11-26NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>1/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>8/11-26NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
</tr>
<tr>
<td>1/11-2NL Not disinfected 80.0 ef</td>
<td>5/11-7NL Not disinfected</td>
<td>40.0 be</td>
<td>8/11-7NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>1/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>8/11-26NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>1/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>8/11-26NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
</tr>
<tr>
<td>1/11-3NL Not disinfected 90.0 f</td>
<td>5/11-6NL Not disinfected</td>
<td>40.0 be</td>
<td>8/11-6NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>1/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>8/11-26NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>1/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>8/11-26NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
</tr>
<tr>
<td>1/11-4NL Not disinfected 60.0 cde</td>
<td>5/11-5NL Not disinfected</td>
<td>40.0 be</td>
<td>8/11-5NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>1/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>8/11-26NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>1/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>8/11-26NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
</tr>
<tr>
<td>1/11-5NL Not disinfected 60.0 cde</td>
<td>5/11-4NL Not disinfected</td>
<td>40.0 be</td>
<td>8/11-4NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>1/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>8/11-26NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>1/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>8/11-26NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
</tr>
<tr>
<td>1/11-6NL Not disinfected 60.0 cde</td>
<td>5/11-3NL Not disinfected</td>
<td>40.0 be</td>
<td>8/11-3NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>1/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>8/11-26NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>1/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>8/11-26NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
</tr>
<tr>
<td>1/11-7NL Not disinfected 60.0 cde</td>
<td>5/11-2NL Not disinfected</td>
<td>40.0 be</td>
<td>8/11-2NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>1/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>8/11-26NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>1/11-5NL Not disinfected</td>
<td>60.0 ab</td>
<td>8/11-26NL Not disinfected</td>
<td>23.3 a-c</td>
<td>3/11-5NL Not disinfected</td>
<td>60.0 ab</td>
</tr>
</tbody>
</table>

*The Alternaria alternata isolate Albas. 1/10 (GenBank Accession number HQ540552) from infected leaves of basil cv. Genovese.*
tained by seeds are virulent denotes the presence of saprophytic strains. By disinfecting seeds with sodium hypochlorite infections by *Alternaria* spp. are only reduced, thus indicating that the pathogen can be present on the surface of seeds, but also internally. The level of infection observed, also at the lower level, seems capable of determine outbreaks of the disease. Seed infection is common in the genus *Alternaria* (Rotem, 1994). Neergaard (1977) listed 36 seedborne *Alternaria* species, which might suggest that practically all species pathogenic to foliage also infect seeds (Rotem, 1994). In some cases (i.e carrot seeds) the pathogen is present on the seed surface, implying contamination rather than infection (Rotem, 1994). In other cases (i.e. cotton) the pathogen penetrates the capsule (Cauquil and Ranney, 1969). The level of seed infection has been evaluated in many host-pathogen combinations: for instance as little as 1% seed infection by *Alternaria carthami* in sunflower resulted in severe outbreaks in commercial fields in Australia (Jackson et al., 1987). In the case of cichory and endive, Barreto et al. (2008) reported seed infection by *Alternaria cichorii*, at levels ranging from 0.6 to 13.75%. Even a low incidence of infection can cause severe crop losses.

*Alternaria* leaf spot represents a potential threat to basil production in Italy as well as in other production areas. The disease has been detected on the most appreciated variety for fresh consumption and pesto production. The pathogen has been isolated from all tested varieties, both commercial and experimental lines of basil. Identifying the primary source of inoculum is of critical importance for effective disease management.

Further research should be carried out to determine the epidemiological significance of seedborne inoculum as well as efficient methods to eliminate the threat to basil production. Seed treatments should take into account the fact that at least two other important pathogens, *Fusarium oxysporum* f. sp. *basilici* and *Peronospora belbahrii* are seed transmitted (Martini et al., 1991; Garibaldi et al., 2004b). While the use of certified pathogen-free propagation material will become an essential qualification for worldwide distribution of this crop, seed dressing with admitted and effective fungicides or with other products should represent an important option for disease control within an integrated disease management approach. All methods should take into account the possible contamination of seeds by different pathogens.

Since the conventional pathogen detection techniques may lack the sensitivity required to detect seedborne pathogens, the detection threshold of *Alternaria* spp. in basil seeds could be enhanced by using molecular techniques, such as polymerase chain reaction, which has proven useful in the case of *Fusarium* wilt on the same crop (Chiocchetti et al., 1999, 2001; Pasquali et al., 2006).

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